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(54) **Thermal cycling reaction apparatus and reactor therefor**

Vorrichtung zur Durchführung von Reaktionen mittels thermischen Zyklen und Reaktor hierfür

Dispositif d'exécution des réactions à cycles thermiques et réacteur correspondant

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XP000444699 WILDING P ET AL: "PCR IN A
SILICON MICROSTRUCTURE"

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Description

[0001] The present invention relates to a thermal cycling reaction apparatus which is useful for polymerase chain reactions (PCR) and other thermal cycling reactions, and to a reactor (reaction vessel) suitable therefor.

Description of the Related Art:

[0002] The PCR technique for a target DNA sequence is known in which a specific target gene is amplified in a large amount in a short time by annealing two kinds of primers respectively to the ends of the target DNA fragment, and repeating a template-specific DNA synthesis reaction with a DNA polymerase in vitro (Japanese Patent Publications 4-67957, 4-67960, etc.). This technique makes it practicable to detect a DNA or a DNA-containing microorganism existing only few in number. Therefore, the PCR technique is widely employed in various technical fields such as biochemistry, biology in a broad sense including genetic engineering, medical science, pharmacology, and agriculture.

[0003] The PCR method, generally, is employed for amplifying a DNA from few number to a larger number by repeating many times a cycle of heating and cooling e.g. a three-step thermal profile (raising and lowering of the temperature) including the first step of keeping a DNA having a targeted DNA sequence at a dissociation temperature (or denaturation temperature) to dissociate the double-stranded DNA into a single-stranded DNA; the second step of keeping the single-stranded DNA at an annealing temperature to anneal thereto a normal-directional primer and a reverse-directional primer; and the third step of keeping the reaction liquid at a temperature for complementary DNA synthesis to grow sequentially the DNA complementary to the single-stranded DNA.

[0004] The PCR is conventionally conducted by use of a computer-controlled automatic temperature-cycling apparatus (a thermal cycler). In an example, an apparatus equipped with such a thermal cycler comprises a metal block which has a bath (cavity) for a holding reaction chamber containing therein a reaction mixture, and a high-temperature fluid storage vessel and a low-temperature fluid storage vessel connected to flow paths to circulate a heating fluid through the metal block. Thereby the temperature of the reaction mixture is automatically changed successively through the aforementioned three steps of prescribed temperatures by switching over the flows of the high-temperature fluid and the low-temperature fluid introduced into the path in the above metal block: for example, at 90-95°C for about 20 seconds in the first step (denaturation), at 45-60°C for about 20 seconds in the second step (annealing), and 65-75°C for about 30 second in the third step (DNA synthesis).

[0005] In another example of the thermal cycler, not for PCR, 100 test-tube type reaction chambers, for in-

stance, which are hung from a rack are transferred successively to five thermostats holding a heating medium of different temperatures, and are dipped therein to conduct a desired enzymatic reaction, enzyme deactivation, or other enzymatic cycling reactions in the respective thermostats (Japanese Patent Publication 62-12986).

[0006] The aforementioned thermal cycler, which changes the temperature of the reaction mixture for the respective steps by raising or lowering the temperature of the heating bath medium in the metal block by switching the circulation of temperature-controlling fluids, has disadvantages as follows. The simple switchover of a heating medium of the temperature for the one step to another heating medium of the temperature of the succeeding step, for example from 90°C for the first step to 45°C for the second step, results in a significantly low rate of temperature change in comparison with the time for the intended reaction, and repetition of the cycles in many times requires extremely long time for the entire treatment. Further, the reaction of the first step (also of the second step) proceeds not only at the set temperature (90°C) but also in a temperature range of several degrees centigrade around the set temperature, which renders it difficult to control the reaction in the prescribed time. In an extreme case, the reaction does not proceed at all, disadvantageously.

[0007] In order to change the temperature quickly for the subsequent step, for example in the above case, a fluid at a temperature of sufficiently lower than the prescribed temperature of the second step can be circulated to the bath, and later circulate a fluid corresponding to the prescribed temperature. In such a method, the temperature of the reaction mixture is liable to become lower than the prescribed temperature to cause so-called overshooting at the end stage of cooling from 90°C to 45°C. This will impair the reproducibility of the reaction, and in an extreme case, the process does not proceed, disadvantageously. Moreover, this method requires additionally a thermostat, a fluid storage vessel, and piping for the high-temperature or low-temperature fluid, which renders it difficult to miniaturize the apparatus, and is not suitable for simultaneous treatment of many samples.

[0008] On the other hand, the latter of the aforementioned systems, in which test tubes hung from a rack are successively delivered and immersed into plural thermostats holding fluids of different set temperatures, requires a mechanical means for delivering and immersing the test tubes, whereby the apparatus becomes larger, and the rapid temperature changes is not readily achievable between the prescribed temperatures.

[0009] Clinical Chemistry Vol. 40, No. 9, 1st September 1994 pages 1815-1818 "PCR in a silicon microstructure" discloses a reactor having a reactor body in the form of a thin plate affording a heat transfer area on at least one face thereof, and the reactor body having etched in the thickness of the plate a cavity forming a

reaction chamber having an opening in at least one face of the thin body and Pyrex glass heat resistant sealing means for sealing the said opening.

[0010] EP-A-318255 discloses thermal cycling apparatus involving cuvettes and a delivery path for the cuvettes which is not linear.

[0011] DE. Gebrauchsmuster G8813773.2 discloses a cylindrical reaction vessel and the transport holder for the reaction vessel is located between the reaction vessel and the temperature controlling block. The reaction vessel does not have a window through which its contents can be observed.

Summary of the Invention:

[0012] The present invention has been achieved to overcome the above disadvantages of conventional thermal cyclers employed in thermal cycling reactions by employing a novel reaction chamber for use in thermal cycling reaction apparatus and novel apparatus in which the chamber can be used.

[0013] A first object of the present invention is to provide a thermal chamber for thermal cycling reaction apparatus which allows rapid temperature changes through prescribed temperature steps to shorten the time of a repeated thermal cycling reaction.

[0014] A second object of the present invention is to provide a thermal cycling reaction apparatus which is capable of keeping the entire reactor at a uniform temperature and avoiding the disadvantage of nonuniformity, in simultaneous treatment of plural samples under the same conditions, in the amount of the reaction product and the reaction progress, independently of the location of the reaction chambers in the reactor, and to provide a reactor suitable therefor.

[0015] A third object of the present invention is to provide a thermal cycling reaction apparatus which is capable of raising or lowering the reaction liquid temperature to a prescribed temperature without overshooting, and enables easy control or omission of a temperature controller, and to provide a reactor suitable therefor. Thereby, the precision of control of the temperature and time of reaction is improved.

[0016] A fourth object of the present invention is to provide a thermal cycling reaction apparatus which can be miniaturized by miniaturizing the temperature controller for the reaction liquid by employing a smaller amount of a reaction liquid sealed in a smaller chamber, and to provide a reactor suitable therefor.

[0017] A fifth object of the present invention is to provide a thermal cycling reaction apparatus for a PCR process which repeats many times a temperature change cycle comprising successive steps of keeping a reaction liquid at a first temperature for dissociating or denaturing a double-stranded DNA having a target DNA sequence into a single-stranded DNA; keeping it at a second temperature for bonding or annealing a normal-directional primer and a reverse-directional primer to the

resulting single-stranded DNA; and keeping it at a third temperature for synthesizing another DNA sequence complementary to the single-stranded DNA in the presence of a DNA polymerase, and to provide a reactor therefor. Thereby, the pre-heating or pre-cooling of the PRC reaction liquid in each step can be substantially omitted to shorten the overall reaction time, and the reaction can be allowed to proceed in a completely sealed chamber to avoid the PCR products and to avoid the contamination caused from aerosol amplified DNA.

[0018] Thus according to the present invention there is provided a reactor comprising a reactor body in a shape of a thin plate, having a heat-transferring area on at least one face of the thin plate adapted to be brought into face-to-face contact with temperature-controlling surfaces, and having one or more cavities of a small volume as a reaction chamber in the thickness of the thin plate, the cavity having an opening on one face or both faces of the thin plate; and a heat-resistant sealing sheet for sealing the reaction chamber by covering the opening of the chamber, characterised in that the reactor body is supported in a thin delivery assisting member composed of material of poor thermal conductivity, so that the reactor body and delivery assisting member are in the form of a thin plate.

[0019] Preferably the sealing means for the reactor comprise a transparent heat resistant sheet.

[0020] The thin plate of the reactor preferably has a thickness of 0.2 to 3mm.

[0021] The reactor preferably has a sealable hole for introducing liquid into the reaction chamber.

[0022] The invention also extends to apparatus for carrying out a thermal cycling reaction comprising a reactor having a reactor body in the form of a thin plate affording a heat transferring area on at least one face thereof, the reactor body defining at least one cavity in the thickness of the plate which cavity affords a reaction chamber which has an opening in one or both faces of the thin plate and heat resistant sealing means for sealing the said opening, means defining a delivery path for the reactor, a plurality of means for controlling the temperature of the reactor body spaced apart along the delivery path, means for moving the reactor along the delivery path and into heat exchange relationship with the said temperature controlling means in turn, and means for controlling the temperature of the said temperature controlling means the reactor being a reactor in accordance with the present invention.

[0023] Preferably optical means are provided for observing the contents of the reaction chamber.

[0024] The temperature controlling means for controlling the temperature of the reactor body are preferably blocks and the reactor is brought into heat conductive contact therewith.

[0025] The means for controlling the temperature of the temperature controlling means may be heaters and sensors.

[0026] In a preferred form of the invention the appa-

ratus is characterised in that the delivery path and the means for moving the reactor along the said delivery path provide heat flow stopping positions opposite the said temperature controlling means at which location the reactor body can be heated or cooled by the temperature controlling means at that stopping position, and one or more observation stopping positions which are spaced from the said temperature controlling means and at which optical observing means are provided for observing the contents of the reaction chamber in the reactor body when it is located at the said observation stopping position.

[0027] The invention also extends to a method of carrying out a PRC reaction characterised in that first, second, and third temperature-controlling surfaces are provided, the first temperature-controlling surface is kept at a dissociation temperature (or denaturation temperature) for a DNA having a target DNA sequence to dissociate the double-stranded DNA into a single-stranded DNA, the second temperature-controlling surface is kept at an annealing temperature for the single-stranded DNA to anneal thereto a normal-directional primer and a reverse-directional primer, and the third temperature-controlling surface is kept at a temperature for complementary DNA synthesis to grow sequentially the DNA complementary to the single-stranded DNA; and delivering a reactor in accordance with the invention in which the cavity contains the PCR reaction mixture into contact with the first, second, and third temperature-controlling surfaces intermittently, and repeating this cycle a number of times.

[0028] In a preferred form of the method at least a fourth position is provided remote from the first, second and third surfaces and optical means are provided for observing the contents of the reaction chamber at the said fourth position and the contents are so observed.

Brief Description of the Drawing:

[0029] Figs. 1A to 1C show schematically a reactor of the present invention used for a thermal cycling reaction. Fig. 1A is a plan view of the reactor, Fig. 1B is a sectional view of the reactor taken along line A-A in Fig. 1A, and Fig. 1C is an enlarged view of the portion B in Fig. 1B.

[0030] Fig. 2 is a schematic sectional front view of an example of the thermal cycling reaction apparatus of the present invention employing the reactor shown in Fig. 1.

[0031] Fig. 3 is a right-hand side view of the apparatus shown in Fig. 2 taken along line C-C.

[0032] Fig. 4 is a bottom end view of the apparatus shown in Fig. 2 taken along line D-D.

[0033] Figs. 5A and 5B are schematic diagrams showing the successive stopping positions of the reactor in a thermal cycling reaction and optical measurement with the reaction apparatus of Fig. 2. Fig. 5A shows the stopping positions during the cycling reaction, and Fig. 5B shows the stopping positions for the optical

measurement.

Detailed Description of the Preferred Embodiment;

5 [0034] The characteristics of the present invention is set forth in the claims.

[0035] The reactor of the present invention employed for a thermal cycling reaction is delivered, along a delivery path having plural and separate temperature-controlling blocks fixed thereon and having respectively a fixed temperature-controlling surface of a prescribed area at a controlled temperature, to contact successively with the temperature-controlling blocks in a predetermined order repeatedly; the reactor comprising a reactor body in a shape of a thin plate, having a heat-transfer area on at least one face of the thin plate to be brought into face-to-face contact with the respective temperature-controlling surfaces, and having a cavity of a small volume as a reaction chamber in the thickness of the thin plate having an opening on one face or both faces of the thin plate; and a heat-resistant sealing sheet for sealing the reaction chamber by covering the opening of the chamber.

[0036] In the above constitution, when an optical detection system is employed to detect the change in the reaction liquid, the sealing sheet for the reaction chamber is preferably a transparent heat-resistant sheet to form a light-transmitting window.

[0037] The aforementioned reactor may be in a shape of a thin plate of a heat-conductive material such as aluminum and other metals, or may be constituted of combination of the above reactor body made of the heat-conductive material with a delivery-assisting member made of a poor heat-conductive material such as nylon, polycarbonate, and other plastic materials. The delivery-assisting member of a poor heat-conductive material is combined to form a thin plate with the reactor body in its entirety. The shape of the delivery-assisting member may be selected to have a shape or structure suitable for the type of the delivery means. For example, the reactor body may be combined with a surface of the delivery-assisting member in a rotor or drum shape to be delivered by rotation of the rotor or the drum. The thin plate-shaped reactor body made of a heat-conductive material is fitted and fixed into a recess or an opening formed on the delivery-assisting member in a plate shape. Naturally, a member may be employed or an operation may be conducted for fixing the reactor.

[0038] The heat-conductive material suitable for constructing the reactor in the present invention includes the materials which have a sufficient thermal conductivity for bringing the reaction chamber quickly to an intended temperature level, preferably having a thermal conductivity of not lower than 20 kcal/m·h·°C such as metallic materials like the aforementioned aluminum. On the other hand, the poor heat-conductive material (heat-insulating material) for constructing the delivery-assisting member includes the materials which have a

sufficiently low thermal conductivity for maintaining the temperature of the reactor body, preferably having a thermal conductivity of not higher than 0.5 kcal/m-h. $^{\circ}$ C such as plastic materials like the aforementioned polycarbonate.

[0039] The reactor body may be formed into any suitable shape as desired, such as a rectangular plate, a circular plate, a flat plate, and a curved plate to be fitted to a drum surface. The size of the reactor is not limited. Generally, the thickness ranges preferably from 0.2 to 3 mm, more preferably from 0.2 to 2 mm for rapid temperature change of the reaction liquid and uniform temperature distribution therein. When the reactor is in a rectangular plate shape, the width ranges preferably from 20 to 40 mm, and the length ranges preferably from 50 to 100 mm.

[0040] The reaction chamber in the reactor body is formed as a cavity in the thickness of the plate. Generally the chamber is a bottomed hole having an opening on the one face of the thin plate, or a through hole piercing the plate for ease of working of the reactor. The opening or openings are sealed liquid-tight against the outside air with a sealing sheet. The opening is generally in a shape of a circle of a diameter ranging from 10 to 20 mm, preferably from 14 to 18 mm, but is not limited thereto. The reaction chamber may be provided singly or in plurality separately in one reactor. The volume of the chamber is about 0.1 mL, preferably in the range of from 0.01 to 0.2 mL for rapid temperature change.

[0041] The sealing sheet for sealing the opening of the reaction chamber may be made of any material which has sufficient resistance to heat, chemicals, and so forth, and does not cause deformation of the sheet or elution of an impurity therefrom. In particular, for optical measurement of the results of the reaction, preferably employed is a sheet transparent or at least transparent at the measurement wave length of a material such as an acrylic resin, polyethylene, and a vinyl chloride resin. The sheet may be a flexible film or a rigid plate.

[0042] The reactor body preferably has a hole for filling the reaction liquid. In particular, the filling hole has a structure to ensure the sealing after filling of the reaction liquid. Examples of the suitable structure of the filling hole include a filling pathway at the side face of the reactor body and heat-sealable after filling of the liquid; a sealable one-way valve allowing liquid filling only; and a rubber plug for filling the liquid by an injection needle and capable of restoring the liquid-tight state after removing the needle.

[0043] The reaction chamber is formed in a heat-transferring area of the reactor body in order to bring the reaction chamber into direct contact with a fixed temperature-controlling surface. The portion to be contacted with the fixed temperature-controlling surface may be at the chamber opening side or at the side having no chamber opening of the reactor body. The heat-transferring area may be provided on one face of the reactor body

or may be provided on both faces of the reactor body in order to be contacted with the fixed temperature-controlling surfaces provided in a pair on both sides of a delivery path. The heat-transferring area is designed to be sufficient to transfer rapidly the heat between the fixed temperature-controlling surface and the entire reactor body. The size of the heat-transferring area to be contacted with the fixed temperature-controlling source is not specially limited. Usually one entire face of the reactor body, or a limited area around the reaction chamber is brought into face-to-face contact with the fixed temperature-controlling surface.

[0044] The feature of the thermal cycling reaction apparatus of the present invention is described below. The thermal cycling reaction apparatus comprises a reactor body constituted of a thin plate of heat-conductive material having a cavity with at least one opening sealed by a sealing sheet on a surface of the reactor, the reactor body supported by delivery-assisting member; a delivery path for guiding the reactor; plural temperature-controlling blocks placed apart from each other so as not to cause thermal interaction along the delivery path and having respectively a fixed temperature-controlling surface of a prescribed area to be brought into contact with the reactor; a temperature-controlling means for maintaining the temperature-controlling surfaces of the temperature-controlling blocks at respectively prescribed temperatures; and a driving means for delivering and stopping the reactor to come into contact with each of the fixed temperature-controlling surfaces of the temperature-controlling blocks in a predetermined order repeatedly.

[0045] As an additional feature, the thermal cycling reaction apparatus of the present invention may further comprise an optical detecting means for detecting optically the change in the reaction chamber, such as a degree of progress of the reaction, through the aforementioned transparent sealing sheet by stopping the reactor at a position other than the temperature-controlling block positions. With this constitution, the optical detecting means enables monitoring of the progress of the reaction with lapse of time, or measuring optically the state of the reaction mixture after the end of the reaction.

[0046] The optical detecting means for detecting optically the change in the reaction liquid includes known conventional optical means such as the one which introduces light reflected by a half mirror into the reaction chamber and observes the light reflected from the chamber through the half mirror visually, or by a light-receiving means like an optical sensor, or an image pick-up means such as a video camera, but is not limited thereto.

[0047] The delivery path for guiding the movement of the reactor in the apparatus of the above constitution is typically a linear path for carrying the reactor linearly in a reciprocating manner by employing a device such as a guide rail, and a guide roller. Otherwise, the delivery path may be a circular or arc-shaped path for rotating

the reactor around an axis by using a rotor type or a drum type of delivery-assisting member.

[0048] The fixed temperature-controlling surface of the above apparatus is formed as a surface of the temperature-controlling block so as to come into contact with the heat-transferring area provided on the one or both faces of the reactor. The temperature-controlling surface is not limited in its shape, and may be planar, curved, rugged, or in any other shape, provided that the surface is capable of coming into close contact with the heat-transferring area. The material for the temperature-controlling block includes metals, plastics, rubbers, ceramics, and the like, and is not specially limited. However, the material and the structure are preferred which has sufficient heat capacity so as to avoid large temperature change by heat exchange on contact with the reactor. The fixed temperature-controlling surfaces are separated so as not to interact thermally with each other. For this purpose, the distance between the surfaces may be kept larger, or a heat-insulating plate may be provided between the temperature-controlling blocks. For ensuring close contact between the fixed temperature-controlling surface of the temperature-controlling block and the reactor, and for ensuring smooth delivery of the reactor, a certain gap is preferably provided between the reactor, and the fixed temperature-controlling surface during delivering the reactor, and the reactor is pushed against the fixed temperature-controlling surface at the time of stopping by a pressing means such as a cylinder mechanism. The temperature-controlling blocks are placed on one side of the delivery path in the case where the reactor is brought into contact with them on one face, or are placed in pairs on both sides of the delivery path in the case where the reactor is brought into contact with them on both faces.

[0049] The temperature-controlling means may be of any type of electric heating, circulation of a heating liquid medium, and the like. Of these, the electric heating is preferred in simplicity and for miniaturization of the apparatus. The temperature control may be conducted to maintain an intended constant temperature by use of a sensor like a thermal sensor by on-off control of the heating source.

[0050] The driving means for delivering the reactor may be constructed, for example, from combination of devices comprising a delivering device such as a roller for delivery of the reactor along the delivery path provided by the guiding device; a driving device for driving the delivering device such as a roller for driving and stopping it to deliver and stop the reactor at the prescribed positions; and a drive-controlling device for controlling the drive according to a sequence program or the like following a prescribed steps. The thermal cycling reaction can be automated and mechanized by employing an MPU (microprocessor unit) for the drive-controlling means.

[0051] The thermal cycling reaction apparatus of the present invention, as described above, is useful for PCR

or the like reactions. Specifically, the first, second, and third fixed temperature-controlling surfaces are provided. The first temperature-controlling surface is kept at a dissociation temperature (or denaturation temperature) of a DNA having a target DNA sequence to dissociate the double-stranded DNA into a single-stranded DNA. The second temperature-controlling surface is kept at an annealing temperature for the single-stranded DNA to anneal thereto a normal-directional primer and a reverse-directional primer. The third temperature-controlling surface is kept at a temperature for complementary DNA synthesis to grow sequentially the DNA complementary to the single-stranded DNA. The delivering means is constructed to deliver the reactor intermittently to the first, second, and third fixed temperature-controlling surfaces. This cycle of the steps is repeated a number of times. Thus the PCR can be readily and surely conducted.

[0052] The PCR conducted according to the present invention is not limited to the above-mentioned type of reaction. Various modifications of PCR can be conducted with the thermal cycling reaction apparatus and reactor of the present invention. For example, a two-temperature PCR, namely a simplified PCR in which the annealing of the primers and synthesis by DNA polymerase are conducted at the same temperature, and denaturation is conducted at a higher temperature, can be conducted by arranging temperature-controlling blocks corresponding to the respective temperatures with the apparatus and the reactor of the present invention.

[0053] Thermal cycling reactions other than the PCR, for example the enzymatic cycling reaction mentioned before (Japanese Patent Publication 62-12986), can be conducted with the reactor and the thermal cycling reaction apparatus of the present invention.

[0054] According to the present invention, the reaction liquid sealed in the reaction chamber formed in the thickness of a thin plate is brought into contact successively with the surfaces of plural temperature-controlling blocks kept at prescribed temperatures, and by this contact, the temperature of the reaction liquid is controlled precisely by the fixed surfaces of the temperature-controlling blocks.

[0055] The typical thermal cycling reaction apparatus for PCR is explained below by reference to the drawings.

[0056] Figs. 1A to 1C show schematically the reactor of the present invention used for a thermal cycling reaction. Fig. 1A is a plan view, Fig. 1B is a sectional view of the reactor taken along line A-A in Fig. 1A, and Fig. 1C is an enlarged sectional view of the portion B in Fig. 1B.

[0057] In the drawings, the reactor 1 comprises a delivery-assisting member 2 made of a heat-insulating acrylic resin in a shape of a rectangular plate, and a reactor body 3 made of heat-conductive aluminum in a shape of a rectangular plate and is fitted to a through hole 201 of the delivery-assisting member 2. The planar

rectangular through hole 201 is formed at a position deviating in a length direction (lateral in Fig. 1) from the center of the member (rightward in Fig. 1), where the reactor body 3 is fitted. The reactor body 3, in this example, has three independent reaction chambers 301 in a shape of bottomed (flat bottomed) round recess (empty space). The one face of the reactor body 3 is covered entirely with a sealing sheet 302 made of a transparent heat-resistant polyethylene to seal the reaction chambers 301. In this example, the delivery-assisting member 2 of the reactor 1 is 130 mm in length, 85 mm in width, and 1.5 mm in thickness; the reactor body 3 is 25 mm in length, 70 mm in width, and 1.5 mm in thickness; and the reaction chamber 301 is 8 mm in radius, and 1 mm in depth, and a small volume of 0.2 mL.

[0058] In this example, the reaction liquid is filled into the reaction chambers 301 of the thin plate reactor 1, and then the sealing sheet 302 is placed thereon, and heat-sealed to enclose the reaction liquid.

[0059] Figs. 2 to 4 illustrates schematically an example of a thermal cycling reaction apparatus. On a lateral face of a casing 5 in a flat box shape, a slit-shaped gateway 501 and a gateway guide 502 are provided at a predetermined height for introducing and removing a reactor. Near the inside wall opposite to the gateway guide 502, a leading guide 503 is provided at the same height as the gateway guide 502. Between the gateway guide 502 and the leading guide 503, a driving roller 504, and driven rollers 505, 506, are placed at prescribed intervals. As can be seen in Figure 4, the rollers 504, 505, 506 are allowed to rotate by pulleys 5041, 5051, 5061 provided at respective ends of the axes of the rollers, and belts 507, 508 put on the pulleys synchronously driven by a motor 509. The internal space is ventilated with a fan 510.

[0060] Four stopping positions 410, 420, 430 and 440 (see Figure 5) are set along the delivery direction of the horizontal linear delivery path defined by the three rollers 504, 505, 506, the gateway guide 502, and the leading guide 503. In this example, successively from the right in Fig. 2, are placed a first temperature-controlling block 6 (position 410), a second temperature-controlling block 7 (position 420), and an open third stopping position 430 between block 7 and a third temperature-controlling block 8. The blocks 6, 7 and 8 are located at the first, second and fourth stopping positions at the upper side of the delivery path, and an optical detector is placed above the third stopping position to measure the change in the reaction chamber. The temperature-controlling means 6 to 8 are constituted respectively by an aluminum block (62, 72, 82) and electric heaters (63, 73, 83) embedded therein, one heater in each block.

[0061] The lower faces of the temperature-controlling blocks 6, 7, 8 afford fixed temperature-controlling surfaces 61, 71, 81 which are formed for contact with the upper face of the reactor 1 so as to keep the reaction liquid as a prescribed temperature in the reaction chamber 301 of the reactor stopped in contact with the block.

In order to achieve close contact between the temperature-controlling surface and the reactor, a slight play in vertical direction may be given to the temperature-controlling block, or downward spring force may be applied to the temperature-controlling block to press it against the reactor, or a vertically directed pressing mechanism may be provided for either or both of them. In this example, the temperature-controlling blocks are spaced apart at intervals of 10 mm or more to avoid thermal interaction between the blocks.

[0062] The temperature of the fixed temperature-controlling surface can be controlled at a prescribed level by a conventional method. In this example, electric heaters (63, 73, 83) are incorporated into the temperature-controlling blocks, (62, 72, 82) and the heaters are turned on and off following the temperature detected by a sensor (64, 74, 84).

[0063] The optical detector 10 provided at the third stopping position (430) comprises a light source 101, a half mirror 102, a lens 103, and a spectrometric filter 104. With this optical detector, the degree of the progress of the reaction in the reaction liquid in the chamber can be monitored visually with lapse of time.

[0064] Fig. 5 is a schematic diagram for explaining an example of operation of the thermal cycling reaction with the above-described apparatus. In Fig. 5, for simplicity, the reactor body 3 only of the reactor assembly is shown.

[0065] In this example, the reactor 1 is brought into face-to-face contact with the first temperature-controlling means 6, which is a block 62 having a fixed temperature-controlling surface 61 kept at a temperature t_2 (90°C) (Step 1- position 410 in Fig. 5). Then the reactor 1 is brought into face-to-face contact with the second temperature-controlling means 7, which is a block 72, having a fixed temperature-controlling surface 71 kept at a room temperature t_1 (Step 2 - position 420 in Fig. 5). Next, the reactor 1 is brought into face-to-face contact with the third temperature-controlling means 8, which is a block 82, having a fixed temperature controlling surface 81 kept at a temperature t_1 (60°) (Step 3 -position 440 in Fig. 5). This cycle of Steps 1 to 3 is repeated N times. After the completion of the N cycles, the reactor 1 is stopped at the detection position 430, and the optical detection is conducted.

[0066] With this reaction apparatus, the reactor 1 is delivered successively to the plural temperature controlling means 6, 7, 8 according to a prescribed sequence program (e.g., for time control) as shown in (a) in Fig. 5. Thereby, the temperature of the reaction liquid can readily be changed to a different temperature state rapidly and kept at that temperature for a prescribed time, and the temperature of the reaction liquid can be controlled stably with high accuracy, advantageously.

[0067] After completion of the reaction cycles, or during the reaction cycles if necessary, the progress of the reaction can be simply measured optically at the third stopping position 430 shown in (b) in Fig. 5.

[0068] The thermal cycling reaction apparatus and the reactor therefor has the following advantages:

(1) The temperature of the reaction liquid can be changed rapidly between plural prescribed temperatures, thereby the time of the repeated cycling reaction can be shortened.

(2) The temperature in the reaction chambers can be made uniform as a whole, and variation among the samples are made smaller.

(3) The temperature of the reaction liquid can be raised or lowered to a prescribed temperature without overshooting, whereby the follow-up control can be facilitated or omitted, and the reaction liquid can readily be controlled to be at a prescribed temperature for a prescribed time with higher accuracy to ensure stable control of the reaction.

(4) The small reactor holding a small amount of a reaction liquid enables miniaturization of the temperature controller, and miniaturization of the entire apparatus.

(5) In practice of PCR, the preheating can be substantially omitted to shorten the reaction time, and further preliminary incorporation of an intercalating fluorescent substance into the reaction liquid prior to PCR allows monitoring of the amplification degree with the reaction chamber completely sealed.

[0069] The invention extends to a reactor for a thermal cycling reaction which is delivered, along a delivery path having plural and separate temperature-controlling blocks fixed thereon and having respectively a fixed temperature-controlling surface of a prescribed area at a controlled temperature, to contact successively with the temperature-controlling blocks in a predetermined order repeatedly: said reactor comprising a reactor body being in a shape of a thin plate, having a heat-transferring area on at least one face of the thin plate to be brought into face-to-face contact with the respective temperature-controlling surfaces, and having a cavity of a small volume as a reaction chamber in the thickness of the thin plate having an opening on one face or both faces of the thin plate; and a heat-resistant sealing sheet for sealing the reaction chamber by covering the opening of the chamber.

[0070] The sealing sheet is preferably a heat-resistant transparent sheet forming a transparent window for optically detecting a change in the reaction chamber from the outside.

[0071] The reactor comprises a delivery-assisting member composed of a poor heat-conductive material, and the reactor body and the delivery-assisting member are formed in a thin plate shape in entirety.

[0072] The reactor may have a sealable liquid-filling hole for filling the reaction liquid into the reaction chamber.

[0073] The reactor is preferably in a shape of a thin plate having a thickness ranging from 0.2 to 3 mm.

[0074] The invention also extends to a thermal cycling apparatus, comprising a reactor as set out above; a delivery path for guiding the reactor; plural temperature-controlling blocks placed apart from each other so as not to cause thermal interaction along the delivery path and having respectively a fixed temperature-controlling surface of a prescribed area to be brought into contact with the reactor; a temperature-controlling means for maintaining the temperature-controlling surfaces of the temperature-controlling blocks at respectively prescribed temperatures; and a driving means for delivery and stopping the reactor to come into contact with each of the fixed temperature-controlling surfaces of the temperature-controlling blocks in a predetermined order repeatedly.

[0075] A transparent heat-resistant sheet is preferably employed for sealing the reactor; a stopping position for the reactor is preferably provided separately from the positions of the temperature-controlling blocks on the delivery path; and an optical detecting means is preferably provided for detecting optically a change in the sealed reactor from the outside through the sealing sheet.

[0076] The plural temperature-controlling blocks are preferably placed separately along the delivery path in a line.

[0077] In a preferred form of the invention the thermal cycling reaction apparatus is for use for PCR, wherein first to third temperature-controlling blocks are provided, the first temperature-controlling block has a fixed first temperature-controlling surface kept at a dissociation temperature (or a denaturation temperature) for a DNA having a target DNA sequence to dissociate the double-stranded DNA into a single-stranded DNA, the second temperature-controlling block has a fixed second temperature-controlling surface kept at an annealing temperature for the single-stranded DNA to anneal thereto a normal-directional primer and a reverse-directional primer, and the third temperature-controlling block has a fixed third temperature-controlling surface kept at a temperature for complementary DNA synthesis to grow sequentially the DNA complementary to the single-stranded DNA; and the delivery means is constructed so as to deliver the reactor intermittently to the first, second and third fixed temperature-controlling surfaces, and repeats this cycle a number of times.

Claims

1. A reactor for a thermal cycling reaction comprising a reactor body (3) in a shape of a thin plate, having a heat-transferring area on at least one face of the thin plate adapted to be brought into face-to-face contact with temperature-controlling surfaces, and having one or more cavities (301) of a small volume as a reaction chamber in the thickness of the thin plate, the cavity having an opening on one face or

- both faces of the thin plate; and a heat-resistant sealing sheet (302) for sealing the reaction chamber by covering the opening of the chamber, **characterised in that** the reactor body (3) is supported in a thin delivery assisting member (2) composed of material of poor thermal conductivity, so that the reactor body (3) and delivery assisting member (2) are in the form of a thin plate.
2. Reactor as claimed in claim 1 **characterised in that** the sealing means for the reactor comprise a transparent heat resistant sheet (302).
 3. A reactor as claimed in claim 1 or claim 2 **characterised in that** the thin plate has a thickness of 0.2 to 3mm.
 4. A reactor as claimed in any one of claims 1 to 3 **characterised in that** the reactor has a sealable hole for introducing liquid into the reaction chamber.
 5. Apparatus for carrying out a thermal cycling reaction comprising a reactor (1) having a reactor body (3) in the form of a thin plate affording a heat transferring area on at least one face thereof, the reactor body defining at least one cavity (301) in the thickness of the plate which cavity affords a reaction chamber which has an opening in one or both faces of the thin plate and heat resistant sealing means (302) for sealing the said opening, means (501, 502, 503) defining a delivery path for the reactor, a plurality of means (6, 7, 8) for controlling the temperature of the reactor body (1) spaced apart along the delivery path, means (504, 505, 506, 5041, 5051, 5061, 507, 508, 509) for moving the reactor (1) along the delivery path and into heat exchange relationship with the said temperature controlling means in turn, and means (63, 73, 83) for controlling the temperature of the said temperature controlling means (6, 7, 8), the reactor being a reactor as claimed in any one of claims 1 to 4.
 6. Apparatus as claimed in claim 5 **characterised in that** optical means (10) are provided for observing the contents of the reaction chamber.
 7. Apparatus as claimed in claim 5 or claim 6 **characterised in that** the temperature controlling means are blocks (62, 72, 82) and the reactor (1) is brought into heat conductive contact therewith.
 8. Apparatus as claimed in claim 5, 6 or 7 **characterised in that** the means for controlling the temperature of the temperature controlling means (6, 7, 8) comprise heaters (63, 73, 83) and sensors (64, 74, 84).
 9. Apparatus as claimed in any one of claims 5 to 8
 10. A method of carrying out a PCR reaction **characterised in that** first, second and third temperature-controlling surfaces (61, 71, 81) are provided, the first temperature-controlling surface (61) is kept at a dissociation temperature (or denaturation temperature) for a DNA having a target DNA sequence to dissociate the double-stranded DNA into a single-stranded DNA, the second temperature-controlling surface (71) is kept at an annealing temperature for the single-stranded DNA to anneal thereto a normal-directional primer and a reverse-directional primer, and the third temperature-controlling surface (81) is kept at a temperature for complementary DNA synthesis to grow sequentially the DNA complementary to the single-stranded DNA; and delivering a reactor (1) as claimed in any one of claims 1 to 4 in which the cavity (301) contains the PCR reaction mixture into contact with the first, second, and third temperature-controlling surfaces intermittently, and repeating this cycle a number of times.
 11. A method as claimed in claim 10 **characterised in that** at least a fourth position is provided remote from the first, second and third surfaces and optical means are provided for observing the contents of the reaction chamber at the said fourth position and the contents are so observed.

45 Patentansprüche

1. Reaktor für eine Thermozyklusreaktion mit einem Reaktorkörper (3) in Form einer dünnen Platte mit einer Wärmeübertragungsfläche auf mindestens einer Seite der dünnen Platte, die geeignet ist, in Seitenberührung mit temperatursteuernden Oberflächen gebracht zu werden, und mit einem oder mehreren Hohlräumen (301) mit einem kleinen Volumen als Reaktionskammer in der Dicke der dünnen Platte, wobei der Hohlraum eine Öffnung auf einer Seite oder beiden Seiten der dünnen Platte hat; und einer wärmebeständigen Dichtungsbahn (302) zum Abdichten der Reaktionskammer durch Abdecken der

- Öffnung der Kammer, **dadurch gekennzeichnet, daß** der Reaktorkörper (3) durch ein dünnes Transporthilfsteil (2) gestützt wird, das aus Material mit schlechter Wärmeleitfähigkeit besteht, so daß der Reaktorkörper (3) und das Transporthilfsteil (2) die Form einer dünnen Platte haben.
2. Reaktor nach Anspruch 1, **dadurch gekennzeichnet, daß** die Dichtungseinrichtung für den Reaktor eine transparente wärmebeständige Bahn (302) aufweist.
 3. Reaktor nach Anspruch 1 oder Anspruch 2, **dadurch gekennzeichnet, daß** die dünne Platte eine Dicke von 0,2 bis 3 mm hat.
 4. Reaktor nach einem der Ansprüche 1 bis 3, **dadurch gekennzeichnet, daß** der Reaktor ein abdichtbares Loch zum Einleiten von Flüssigkeit in die Reaktionskammer hat.
 5. Vorrichtung zum Durchführen einer Thermozyklusreaktion mit einem Reaktor (1) mit einem Reaktorkörper (3) in Form einer dünnen Platte, die eine Wärmeübertragungsfläche auf mindestens einer Seite hat, wobei der Reaktorkörper mindestens einen Hohlraum (301) in der Dicke der Platte bildet, wobei der Hohlraum eine Reaktionskammer bildet, die eine Öffnung in einer oder beiden Seiten der dünnen Platte und eine wärmebeständige Dichtungseinrichtung (302) zum Abdichten der Öffnung hat, Einrichtungen (501, 502, 503), die einen Transportweg für den Reaktor bilden, mehreren Einrichtungen (6, 7, 8) zum Steuern der Temperatur des Reaktorkörpers (1), die auf dem Transportweg beabstandet sind, Einrichtungen (504, 505, 506, 5041, 5051, 5061, 507, 508, 509) zum Bewegen des Reaktors (1) auf dem Transportweg und nacheinander in Wärmetauschbeziehung mit den temperatursteuernden Einrichtungen, und Einrichtungen (63, 73, 83) zum Steuern der Temperatur der temperatursteuernden Einrichtungen (6, 7, 8), wobei der Reaktor ein Reaktor nach einem der Ansprüche 1 bis 4 ist.
 6. Vorrichtung nach Anspruch 5, **dadurch gekennzeichnet, daß** eine optische Einrichtung (10) zum Beobachten des Inhalts der Reaktionskammer vorgesehen ist.
 7. Vorrichtung nach Anspruch 5 oder Anspruch 6, **dadurch gekennzeichnet, daß** die temperatursteuernden Einrichtungen Blöcke (62, 72, 82) sind und der Reaktor (1) in Wärmeleitberührung mit ihnen gebracht wird.
 8. Vorrichtung nach Anspruch 5, 6 oder 7, **dadurch gekennzeichnet, daß** die Einrichtungen zum Steuern der Temperatur der temperatursteuernden Einrichtungen (6, 7, 8) Heizer (63, 73, 83) und Sensoren (64, 74, 84) aufweisen.
 9. Vorrichtung nach einem der Ansprüche 5 bis 8, **dadurch gekennzeichnet, daß** der Transportweg und die Einrichtungen zum Bewegen des Reaktors auf dem Transportweg vorsehen: Wärmefluß-Haltepositionen gegenüber den temperatursteuernden Einrichtungen, wobei an dieser Stelle der Reaktorkörper durch die temperatursteuernden Einrichtungen an dieser Halteposition erwärmt oder abgekühlt werden kann, sowie eine oder mehrere Beobachtungs-Haltepositionen, die von den temperatursteuernden Einrichtungen beabstandet sind und an denen eine optische Beobachtungseinrichtung zum Beobachten des Inhalts der Reaktionskammer im Reaktorkörper vorgesehen ist, wenn er sich an den Beobachtungs-Haltepositionen befindet.
 10. Verfahren zur Durchführung einer PCR-Reaktion, **dadurch gekennzeichnet, daß** eine erste, zweite und dritte temperatursteuernde Oberfläche (61, 71, 81) vorgesehen sind, die erste temperatursteuernde Oberfläche (61) auf einer Dissoziationstemperatur (oder Denaturierungstemperatur) für eine DNA mit einer DNA-Zielsequenz gehalten wird, um die doppelsträngige DNA zu einer einzelsträngigen DNA zu dissoziieren, die zweite temperatursteuernde Oberfläche (71) auf einer Wärmekopplungstemperatur für die einzelsträngige DNA gehalten wird, um einen normal gerichteten Primer und einen rückwärtig gerichteten Primer daran durch Wärme anzukoppeln, und die dritte temperatursteuernde Oberfläche (81) auf einer Temperatur zur komplementären DNA-Synthese gehalten wird, um die DNA komplementär zur einsträngigen DNA sequentiell zu vermehren; sowie durch Intermittieren des Transportieren eines Reaktors (1) nach einem der Ansprüche 1 bis 4, in dem der Hohlraum (301) das PCR-Reaktionsgemisch enthält, in Berührung mit der ersten, zweiten und dritten temperatursteuernden Oberfläche und mehrfaches Wiederholen dieses Zyklus.
 11. Verfahren nach Anspruch 10, **dadurch gekennzeichnet, daß** mindestens eine vierte Position entfernt von der ersten, zweiten und dritten Oberfläche vorgesehen ist und eine optische Einrichtung zum Beobachten des Inhalts der Reaktionskammer an der vierten Position vorgesehen ist und der Inhalt auf diese Weise beobachtet wird.

55 Revendications

1. Un réacteur pour réaction à cycles thermiques comprenant un corps de réacteur (3) à la forme de pla-

- que mince, ayant une surface de transfert de chaleur sur une face au moins de la plaque mince adaptée pour être amenée au contact face à face de surfaces de commande de température, et ayant une cavité (ou plusieurs cavités) (301) de faible volume servant de chambre de réaction dans l'épaisseur de la plaque mince, la cavité ayant une ouverture sur une face ou sur les deux faces de la plaque mince ; et une feuille d'étanchéité (302) résistant à la chaleur pour sceller la chambre de réaction en couvrant l'ouverture de la chambre, **caractérisé en ce que** le corps de réacteur (3) est soutenu dans un élément mince d'aide à l'amenée (2) composé d'un matériau mauvais conducteur de chaleur, de sorte que le corps de réacteur (3) et l'élément d'aide à l'amenée sont sous la forme d'une plaque mince.
2. Le réacteur selon la revendication 1 **caractérisé en ce que** le moyen d'étanchéité pour le réacteur comprend une feuille transparente (302) résistant à la chaleur.
 3. Un réacteur selon la revendication 1 ou 2 **caractérisé en ce que** la plaque mince a une épaisseur de 0,2 mm à 3 mm.
 4. Un réacteur selon l'une quelconque des revendications 1 à 3 **caractérisé en ce que** le réacteur présente un trou scellable pour introduire le liquide dans la chambre de réaction.
 5. Un dispositif pour l'exécution d'une réaction à cycles thermiques comprenant un réacteur (1) ayant un corps de réacteur (3) à la forme d'une plaque mince présentant une surface de transfert de chaleur sur au moins une de ses faces, le corps de réacteur définissant au moins une cavité (301) dans l'épaisseur de la plaque, laquelle cavité présente une chambre de réaction qui a une ouverture aménagée dans une face ou les deux faces de la plaque mince et un moyen d'étanchéité (302) résistant à la chaleur pour sceller la dite ouverture, des moyens (501, 502, 503) définissant une voie d'amenée pour le réacteur, une pluralité de moyens (6, 7, 8) pour commander la température du corps de réacteur (1) espacés l'un de l'autre le long de la voie d'amenée, des moyens (504, 505, 506, 5041, 5051, 5061, 507, 508, 509) pour déplacer le réacteur (1) le long de la voie d'amenée et le placer dans une relation d'échange de chaleur avec ledit moyen de commande de température, et des moyens (63, 73, 83) pour commander la température des dits moyens de commande de température (6, 7, 8), le réacteur étant un réacteur selon l'une des revendications 1 à 4.
 6. Un dispositif selon la revendication 5 **caractérisé en ce que** des moyens optiques (10) sont prévus pour observer le contenu de la chambre de réaction.
 7. Le dispositif selon la revendication 5 ou 6, **caractérisé en ce que** les moyens de commande de température sont des blocs (62, 72, 82) et le réacteur (1) est amené à leur contact conducteur de chaleur.
 8. Le dispositif selon la revendication 5, 6 ou 7 **caractérisé en ce que** les moyens pour commander la température des moyens de commande de température (6, 7, 8) comprennent des dispositifs de chauffage (63, 73, 83) et des capteurs (64, 74, 84).
 9. Le dispositif selon l'une quelconque des revendications 5 à 8 **caractérisé en ce que** la voie d'amenée et les moyens pour déplacer le réacteur le long de la dite voie d'amenée présentent des positions d'arrêt du flux de chaleur en face des dits moyens de commande de température à l'emplacement duquel le corps de réacteur peut être chauffé ou refroidi par les moyens de commande de température à cette position d'arrêt ; et une ou plusieurs positions d'arrêt d'observation qui sont séparées des dits moyens de commande de température et en lesquelles des moyens optiques d'observation sont prévus pour observer le contenu de la chambre de réaction dans le corps de réacteur lorsqu'il est placé à la dite position d'arrêt d'observation.
 10. Un procédé d'exécution d'une réaction PCR **caractérisé en ce qu'**une première, deuxième et troisième surfaces de commande de température (61, 71, 81) sont prévues, la première surface de commande de température (61) est maintenue à une température de dissociation (ou température de dénaturation) pour un ADN ayant une séquence d'ADN cible afin de dissocier l'ADN double brin en un ADN simple brin, la deuxième surface de commande de température (71) est maintenue à une température d'anneau pour que l'ADN simple brin y renature une amorce à sens normal et une amorce à sens inverse, et la troisième surface de commande de température (81) est maintenue à une température pour la synthèse d'ADN complémentaire pour développer séquentiellement l'ADN complémentaire de l'ADN simple brin ; et d'amenée d'un réacteur (1) selon l'une quelconque des revendications 1 à 4 dans lequel la cavité (301) contient le mélange réactionnel de PCR en contact intermittent avec les première, deuxième et troisième surfaces de commande de température, et de répétition à plusieurs reprises de ce cycle.
 11. Un procédé selon la revendication 10 **caractérisé en ce qu'**au moins une quatrième position est prévue à distance des première, deuxième et troisième surfaces, et des moyens optiques sont prévus pour

observer le contenu de la chambre de réaction en la dite quatrième position et le contenu est observé.

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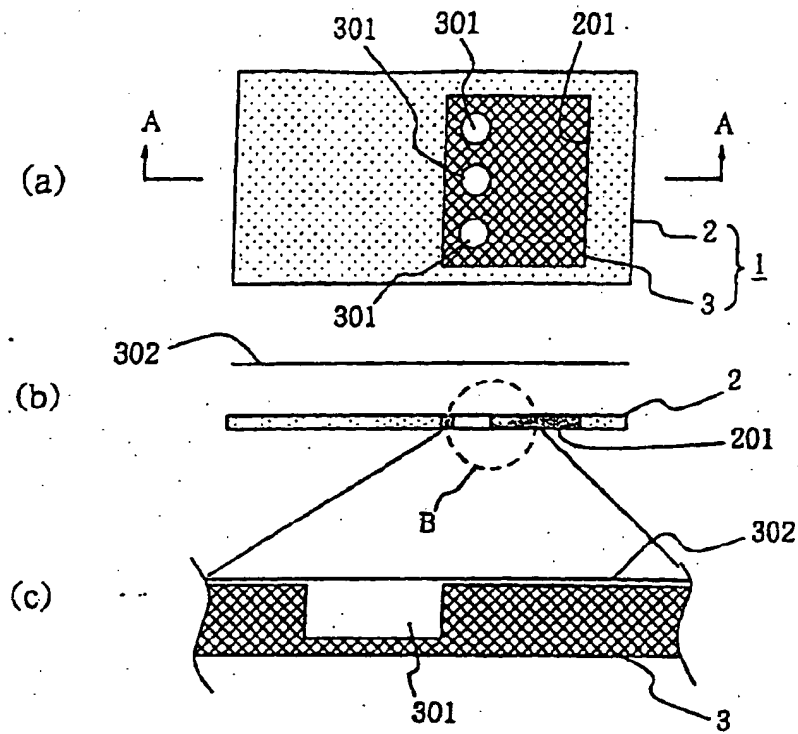
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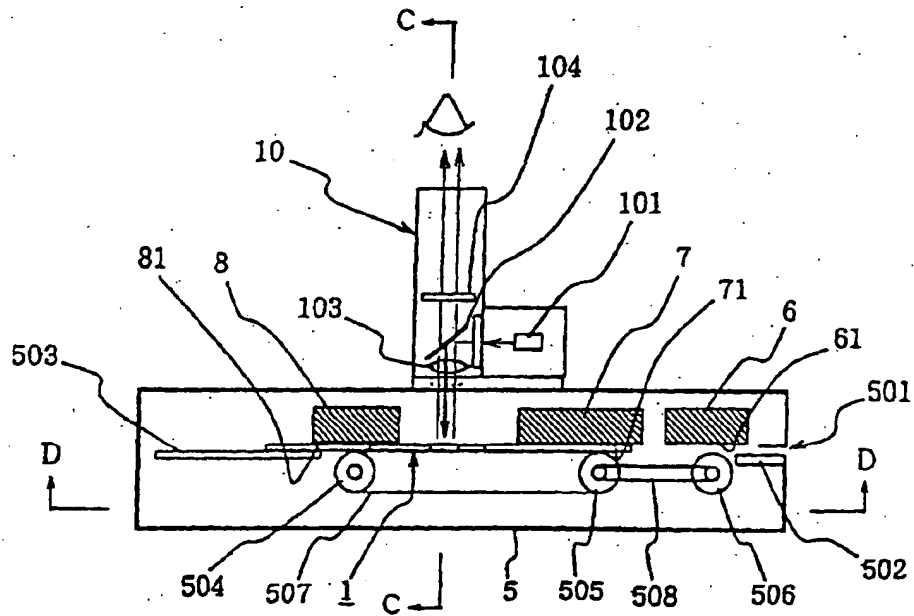
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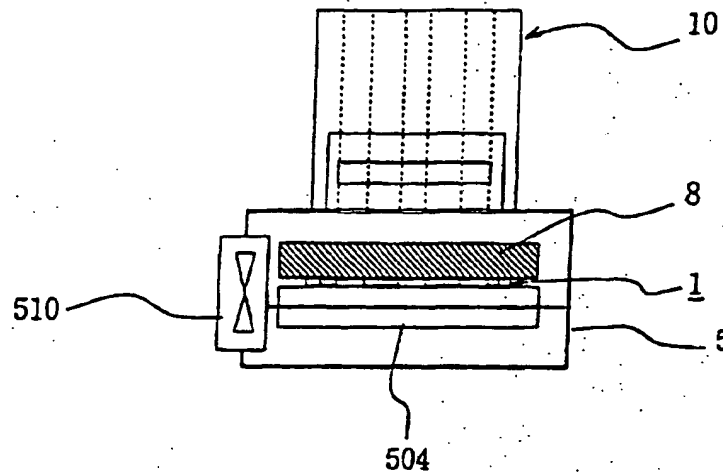
(FIG. 1)



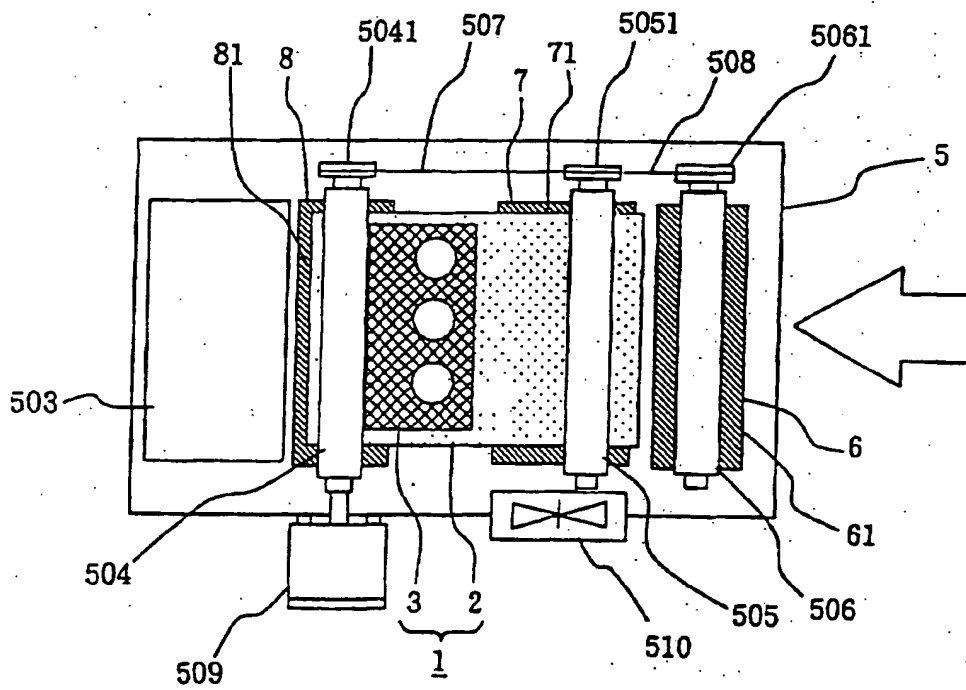
(FIG. 2)



(FIG. 3)



(FIG. 4)



(FIG. 5)

